# WEST Search History

DATE: Thursday, August 28, 2003

| Set Name side by side | Query | Hit Count | Set Name result set |
|---|---|---|---|
| | *DB=USPT,PGPB,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| L1 | ompa same (tissue plaminogen activator or tpa or t-pa or k2s or kringle adj1 2 adj1 serine protease) | 5 | L1 |

END OF SEARCH HISTORY

WEST

Generate Collection | Print

**Search Results** - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 20030049729 A1

L1: Entry 1 of 5 File: PGPB Mar 13, 2003

PGPUB-DOCUMENT-NUMBER: 20030049729
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030049729 A1

TITLE: Methods for large scale production of recombinant DNA-Derived TPA or K2S molecules

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Manosroi, Jiradej | Chiang Mai | | TH | |
| Manosroi, Aranya | Chiang Mai | | TH | |
| Tayapiwatana, Chatchai | BKK | | TH | |
| Goetz, Friedrich | Tuebingen | | DE | |
| Werner, Rolf-Guenther | Biberach | | DE | |

US-CL-CURRENT: 435/69.1; 435/252.33, 435/320.1, 435/488, 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20030013150 A1

L1: Entry 2 of 5 File: PGPB Jan 16, 2003

PGPUB-DOCUMENT-NUMBER: 20030013150
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030013150 A1

TITLE: Methods for large scale protein production in prokaryotes

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Manosroi, Jiradej | Chiang Mai | | TH | |
| Manosroi, Aranya | Chiang Mai | | TH | |
| Tayapiwatana, Chatchai | Bkk | | TH | |
| Goetz, Friedrich | Tuebingen | | DE | |
| Werner, Rolf-Guenther | Biberach | | DE | |

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/455, 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

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☐ 3. Document ID: US 6083715 A

L1: Entry 3 of 5 File: USPT Jul 4, 2000

US-PAT-NO: 6083715
DOCUMENT-IDENTIFIER: US 6083715 A
**** See image for Certificate of Correction ****

TITLE: Methods for producing heterologous disulfide bond-containing polypeptides in bacterial cells

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Georgiou; George | Austin | TX | | |
| Oiu; Ji | Austin | TX | | |
| Bessette; Paul | Austin | TX | | |
| Swartz; James | Menlo Park | CA | | |

US-CL-CURRENT: 435/69.1; 435/252.1, 435/252.8, 435/320.1, 435/69.7, 536/23.1, 536/23.4

ABSTRACT:

Disclosed are methods and compositions for producing heterologous disulfide bond containing polypeptides in bacterial cells. In preferred embodiments the methods involve co-expression of a prokaryotic disulfide isomerase, such as DsbC or DsbG and a gene encoding a recombinant eukaryotic polypeptide. Exemplary polypeptides disclosed include tissue plasminogen activator.

46 Claims, 5 Drawing figures
Exemplary Claim Number: 2
Number of Drawing Sheets: 3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

---

☐ 4. Document ID: US 6027888 A

L1: Entry 4 of 5 File: USPT Feb 22, 2000

US-PAT-NO: 6027888
DOCUMENT-IDENTIFIER: US 6027888 A

TITLE: Methods for producing soluble, biologically-active disulfide-bond containing eukaryotic proteins in bacterial cells

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Georgiou; George | Austin | TX | | |
| Ostermeier; Marc | State College | PA | | |

US-CL-CURRENT: 435/6; 435/243, 435/320.1, 435/69.1, 435/91.1, 530/350, 536/23.2, 536/23.5

ABSTRACT:

Disclosed are methods of producing eukaryotic disulfide bond-containing polypeptides in bacterial hosts, and compositions resulting therefrom. Co-expression of a eukaryotic foldase and a disulfide bond-containing polypeptide in a bacterial host cell is demonstrated. In particular embodiments, the methods have been used to produce mammalian pancreatic trypsin inhibitor and tissue plasminogen activator (tPA) in soluble, biologically-active forms, which are isolatable from the bacterial periplasm. Also disclosed are expression systems, recombinant vectors, and transformed host cells.

40 Claims, 11 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 7



5. Document ID: US 20030049729 A1 WO 200240650 A2 AU 200221815 A

L1: Entry 5 of 5 File: DWPI Mar 13, 2003

DERWENT-ACC-NO: 2002-519376
DERWENT-WEEK: 200321


TITLE: Producing active, correctly folded recombinant tissue plasminogen activator, Kringle 2 serine protease in prokaryotic cells by expressing the protein-encoding DNA operably linked to DNA coding for signal peptide OmpA

INVENTOR: GOETZ, F; MANOSROI, A ; MANOSROI, J ; TAYAPIWATANA, C ; WERNER, R

PRIORITY-DATA: 2000GB-0027779 (November 14, 2000)

PATENT-FAMILY:

| PUB-NO | PUB-DATE | LANGUAGE | PAGES | MAIN-IPC |
|---|---|---|---|---|
| US 20030049729 A1 | March 13, 2003 | | 000 | C12P021/02 |
| WO 200240650 A2 | May 23, 2002 | E | 080 | C12N009/00 |
| AU 200221815 A | May 27, 2002 | | 000 | C12N009/00 |

INT-CL (IPC): C12 N 1/21; C12 N 9/00; C12 N 15/74; C12 P 19/34; C12 P 21/02

ABSTRACTED-PUB-NO: WO 200240650A
BASIC-ABSTRACT:

NOVELTY - Producing (M1) extracellularly secreted, active, correctly folded, recombinant tissue plasminogen activator (tPA) (I), Kringle 2 serine protease molecule (K2S) (II), or their variants (Ia,Ib) in prokaryotic cells (C1) by using a (C1) containing and expressing vector comprising DNA encoding (I,II,Ia or Ib) operably linked to DNA coding for signal peptide OmpA or its functional derivative, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a DNA molecule (III) coding for the OmpA protein or its functional derivative, operably linked to a DNA molecule coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tPA;

(2) a fusion protein (IV) of OmpA and K2S, comprising a fully defined sequence of 377 amino acids (S8) as given in the specification, or its fragment, functional variant,

allelic variant, a subunit, a chemical derivative or a glycosylation variant;

(3) a K2S protein (V) comprising a fully defined sequence of SEGN (S9) or its variant, fragment, functional variant, allelic variant, subunit, chemical derivative, fusion protein or glycosylation variant;

(4) a vector (VI) containing (III);

(5) a vector pComb3HSS (VII) containing (III), where the expression of the gp III protein is suppressed or inhibited by deleting the DNA molecule encoding the gp III protein or by a stop codon between the gene coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tissue plasminogen activator protein and the gp III gene; and

(6) a prokaryotic host cell (VIII) comprising (III), (VI) or (VII).

ACTIVITY - Cerebroprotective; Cardiant; Thrombolytic.

No biological data is given.

MECHANISM OF ACTION - Mediator of fibrin formation and clot dissolution.

USE - M1 is useful for producing recombinant DNA-derived tissue plasminogen activator (tPA), Kringle 2 serine protease molecule (K2S), or variants of tPA or K2S molecule in a prokaryotic cell such as Escherichia coli. (III), (VI), (VII) or (VIII) are used in the method for producing a polypeptide with the activity of tPA protein. Preferably, the molecules are useful in (M1) (all claimed).

The DNA molecules, vectors or host cells are useful for producing a polypeptide having the activity of tissue plasminogen activator. Recombinant DNA-derived polypeptides from (M1) are useful for manufacturing a medicament for treating stroke, cardiac infarction, acute myocardial infarction, pulmonary embolism, any artery occlusion such as coronary artery occlusion, intracranial artery occlusion (e.g., arteries supplying the brain), peripherally occluded arteries, deep vein thrombosis, or related diseases associated with unwanted blood clotting.

ADVANTAGE - The use of the signal peptide OmpA alone and/or in combination with the N-terminal amino acids SEGN (S9)/SEGNSD (S10) translocate the recombinant DNA-derived tPA, tPA variant, K2S molecule or K2S variant to the outer surface and facilitates the release of the functional and active molecule into the culture medium to a greater extent than any other known method. Before crossing the outer membrane, the recombinant DNA-derived protein is correctly folded, the signal peptide is cleaved off to produce a mature molecule and the efficiency of signal peptide removal is very high and leads to correct folding of the recombinant DNA-derived protein.



| Terms | Documents |
|---|---|
| ompa same (tissue plaminogen activator or tpa or t-pa or k2s or kringle adj1 2 adj1 serine protease) | 5 |

```
=> file .nash
=> s ompa and (tissue plasminogen activator or tpa or kringle(1w)2 (1w)serine protease or K2S)
L1            3 FILE MEDLINE
L2            7 FILE CAPLUS
L3            3 FILE SCISEARCH
L4            2 FILE LIFESCI
L5            2 FILE BIOSIS
L6            1 FILE EMBASE

TOTAL FOR ALL FILES
L7           18 OMPA AND (TISSUE PLASMINOGEN ACTIVATOR OR TPA OR KRINGLE(1W) 2
                (1W) SERINE PROTEASE OR K2S)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8            7 DUP REM L7 (11 DUPLICATES REMOVED)

=> d ibib abs 1-7

L8   ANSWER 1 OF 7  CAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:         2002:391899  CAPLUS
DOCUMENT NUMBER:          136:396980
TITLE:                    Methods for large scale protein production in
                          prokaryotes
INVENTOR(S):              Werner, Rolf-Guenther; Goetz, Friedrich; Tayapiwatana,
                          Chatchai; Manosroi, Jiradej; Manosroi, Aranya
PATENT ASSIGNEE(S):       Boehringer Ingelheim International Gmbh, Germany
SOURCE:                   PCT Int. Appl., 52 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
     WO 2002040696      A2   20020523       WO 2001-EP12920  20011108
     WO 2002040696      A3   20020919
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL,
             PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
             US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
             BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
     AU 2002021824      A5   20020527       AU 2002-21824    20011108
     US 2003013150      A1   20030116       US 2001-987457   20011114
PRIORITY APPLN. INFO.:                     GB 2000-27782    A  20001114
                                           US 2001-268573P  P  20010215
                                           WO 2001-EP12920  W  20011108
AB   The invention belongs to the field of protein prodn. in prokaryotic cells.
     The invention relates to methods for the prodn. of recombinant DNA-derived
     heterologous protein in prokaryotic cells, wherein said heterologous
     protein is secreted extracellularly as an active and correctly folded
     protein, and the prokaryotic cell contains and expresses a vector
     comprising the DNA coding for said heterologous protein operably linked to
     the DNA coding for the signal peptide OmpA.

L8   ANSWER 2 OF 7  CAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:         2002:391858  CAPLUS
DOCUMENT NUMBER:          136:400703
TITLE:                    Large scale production and secretion of active
                          recombinant human tissue plasminogen
                          activator derivatives in Escherichia coli
INVENTOR(S):              Werner, Rolf-Guenther; Goetz, Friedrich; Tayapiwatana,
                          Chatchai; Manosroi, Jiradej; Manosroi, Aranya
PATENT ASSIGNEE(S):       Boehringer Ingelheim International Gmbh, Germany
SOURCE:                   PCT Int. Appl., 80 pp.
```

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002040650 | A2 | 20020523 | WO 2001-EP12857 | 20011107 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2002021815 | A5 | 20020527 | AU 2002-21815 | 20011107 |
| US 2003049729 | A1 | 20030313 | US 2001-987455 | 20011114 |
| NO 2003002143 | A | 20030707 | NO 2003-2143 | 20030513 |

PRIORITY APPLN. INFO.: GB 2000-27779 A 20001114
US 2001-268574P P 20010215
WO 2001-EP12857 W 20011107

AB The invention relates to methods for the prodn. of a recombinant **tissue plasminogen activator** (**tPA**), and its variants such as Kringle 2 Serine (**K2S**) deletion mutant, in prokaryotic cells, wherein said **tPA** or **K2S** or variant is secreted extracellularly as an active and correctly folded protein, and the prokaryotic cell contains and expresses a vector comprising the DNA coding for said **tPA** or **K2S** or variant operably linked to the DNA coding for the signal peptide **OmpA**. The DNA fragment coding for **kringle 2** plus **serine protease** domains (**K2S**) of **tissue plasminogen activator** (**tPA**) was inserted into a phagemid vector, pComb3HSS. In the recombinant vector, pComb3H-**K2S**, the **K2S** gene was fused to gpIII of .PHI.M13 and linked to the **OmpA** signal sequence. The resulting gene, rK2S-gpIII, was expressed in Escherichia coli XL-1 Blue. The protein was presented on the phage particle. To stop the expression of gpIII, a stop codon between **K2S** and the gpIII gene was inserted by site-directed mutagenesis. This mutated vector, MpComb3H-**K2S**, was transformed in XL-1 Blue. After induction with IPTG (isopropyl-.beta.-D-thiogalactopyranoside), rK2S was found both in the periplasm as an inactive form of approx. 32% and in the culture supernatant as an active form of approx. 68%. The secreted form of rK2S was partially purified by ammonium sulfate (55%) pptn. The periplasmic form was isolated from whole cells by chloroform extn. The fibrin binding site of kringle 2 was demonstrated in all expressed versions (phage-bound, periplasmic, and secreted forms) using the monoclonal anti-kringle 2 antibody (16/B). Only the secreted form of rK2S revealed a fibrinogen-dependent amidolytic activity with the specific activity of 236 IU/.mu.g. No amidolytic activity of rK2S was obsd. in either the periplasmic or the phage-bound form. The secretion of rK2S as an active enzyme offers a novel approach for the prodn. of the active-domain deletion mutant **tPA**, rK2S, without any requirements for bacterial compartment prepn. and in vitro refolding processes. This finding is an important technol. advance in the development of large-scale, bacterial based **tPA** prodn. systems.

L8 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002107394 MEDLINE
DOCUMENT NUMBER: 21827973 PubMed ID: 11838275
TITLE: Lekteplase--a secreted **tissue plasminogen activator** derivative from Escherichia coli.
AUTHOR: Manosroi Jiradey; Tayapiwatana Chatchai; Manosroi Aranya; Beer Jurgen; Bergemann Klaus; Werner Rolf Gunter
CORPORATE SOURCE: Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.
SOURCE: ARZNEIMITTEL-FORSCHUNG, (2002) 52 (1) 60-6.
Journal code: 0372660. ISSN: 0004-4172.

PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020213
Last Updated on STN: 20020324
Entered Medline: 20020322

AB Fermentation studies of batch-mode cultivation in 4-L fermenters were carried out to obtain an active recombinant DNA-derived **tissue plasminogen activator** (t-PA) deletion mutant, lekteplase, secreted and correctly folded from Escherichia coli. The **OmpA** signal sequence was used to deliver the heterologous product composed of **kringle 2** plus **serine protease** domain (**K2S**) to the medium. Supplementing the complex medium with 10% glycerol and 20 mmol/l magnesium chloride led to an increase in cell numbers with final cell density reaching an OD600 of 24. The expression level of lekteplase in the medium detected by sandwich ELISA was 100 mg/L. Enzymatic activity of lysine-sepharose purified product was demonstrated by amidolytic assay, in vitro fibrin clot lysis, and copolymerization PAGE.

L8 ANSWER 4 OF 7 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001292310 MEDLINE
DOCUMENT NUMBER: 21268862 PubMed ID: 11375177
TITLE: Secretion of active recombinant human **tissue plasminogen activator** derivatives in Escherichia coli.
AUTHOR: Manosroi J; Tayapiwatana C; Gotz F; Werner R G; Manosroi A
CORPORATE SOURCE: Pharmaceutical Cosmetic Raw Materials and Natural Products Research and Development Center, Institute for Science and Technology Research and Development, Chiang Mai University, 50200 Chiang Mai, Thailand.. pmpti006@chiangmai.ac.th
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2001 Jun) 67 (6) 2657-64.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010820
Last Updated on STN: 20021211
Entered Medline: 20010816

AB The DNA fragment coding for **kringle 2** plus **serine protease** domains (**K2S**) of **tissue plasminogen activator** (**tPA**) was inserted into a phagemid vector, pComb3HSS. In the recombinant vector, pComb3H-**K2S**, the **K2S** gene was fused to gpIII of PhiM13 and linked to the **OmpA** signal sequence. The resulting gene, rK2S-gpIII, was inducibly expressed in Escherichia coli XL-1 Blue. The protein was presented on the phage particle. To stop the expression of gpIII, a stop codon between **K2S** and the gpIII gene was inserted by site-directed mutagenesis. This mutated vector, MpComb3H-**K2S**, was transformed in XL-1 Blue. After induction with IPTG (isopropyl-beta-D-thiogalactopyranoside), rK2S was found both in the periplasm as an inactive form of approximately 32% and in the culture supernatant as an active form of approximately 68%. The secreted form of rK2S was partially purified by ammonium sulfate (55%) precipitation. The periplasmic form was isolated from whole cells by chloroform extraction. The fibrin binding site of kringle 2 was demonstrated in all expressed versions (phage-bound, periplasmic, and secreted forms) using the monoclonal anti-kringle 2 antibody (16/B). Only the secreted form of rK2S revealed a fibrinogen-dependent amidolytic activity with the specific activity of 236 IU/microg. No amidolytic activity of rK2S was observed in either the periplasmic or the phage-bound form. The secretion of rK2S as an active enzyme offers a novel approach for the production of the active-domain deletion mutant **tPA**, rK2S, without any requirements for bacterial compartment preparation and in vitro refolding processes. This finding is an important technological advance in the development of large-scale, bacterium-based **tPA** production

systems.

L8 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:196531 CAPLUS
DOCUMENT NUMBER: 132:247149
TITLE: Synthetic operon for a Dsb family of Escherichia disulfide bond-forming enzymes and coexpression of exogenous proteins with increased secretion into periplasm in bacteria
INVENTOR(S): Kurokawa, Yoichi; Yanagi, Hideki; Yura, Takashi
PATENT ASSIGNEE(S): H.S.P. Kenkyusho K. K., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 23 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2000083670 | A2 | 20000328 | JP 1998-255702 | 19980909 |
| EP 992588 | A1 | 20000412 | EP 1999-117806 | 19990909 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: JP 1998-255702 19980909

AB An expression vector contg. a synthetic operon for a family of E. coli disulfide bond-forming enzymes DsbA, DsbB, DsbC, and DsbD and an inducible promoter, and method for expression of exogenous protein in sol. form are claimed. Prodn. of eukaryotic protein in active form, and secretion into periplasm, where the oxidized environment favors formation of correct three-dimensional structure, in prokaryotic host organism such as E. coli, can be achieved. Genes coding for DsbA, DsbB, DsbC, and DsbD were cloned from E. coli, and an arabinose-inducible expression vector contg. a synthetic operon for those genes was constructed. Co-expression of these Dsb family genes with genes for exogenous proteins, **OmpA**/OmpT signal peptide, NGF-.beta., or horse radish peroxidase (HRP) in E. coli resulted in increased secretion of these exogenous proteins into periplasm with increasing arabinose concn.

L8 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000054063 MEDLINE
DOCUMENT NUMBER: 20054063 PubMed ID: 10585186
TITLE: Facilitating the formation of disulfide bonds in the Escherichia coli periplasm via coexpression of yeast protein disulfide isomerase.
AUTHOR: Zhan X; Schwaller M; Gilbert H F; Georgiou G
CORPORATE SOURCE: Institute of Cell and Molecular Biology, Department of Chemical Engineering, University of Texas, Austin, Texas 78712, USA.
SOURCE: BIOTECHNOLOGY PROGRESS, (1999 Nov-Dec) 15 (6) 1033-8.
Journal code: 8506292. ISSN: 8756-7938.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000107

AB Sacchromyces cerevisiae protein disulfide isomerase (yPDI) was expressed in the E. coli periplasm by using plasmids encoding the **OmpA**-yPDI-(His)(6) fusion gene under the control of the araBAD, trc, or T7 promoter. The expression levels of yeast PDI under these promoters were compared. Our results showed that yeast PDI expressed into the periplasm could catalyze the formation of disulfide bonds in alkaline phosphatase, restoring the phoA(+) phenotype in dsbA(-) mutants. The yeast PDI was purified from the Escherichia coli periplasm and shown to exhibit catalytic properties comparable to those of the rat enzyme with reduced RNase as substrate. In vivo, coexpression of the yeast PDI increased the yield of bovine pancreatic trypsin inhibitor (BPTI) in E. coli by 2-fold, similar to the effect seen previously with the coexpression of the rat

enzyme. However yeast PDI was more effective than rat PDI in facilitating the expression of active **tissue plasminogen activator** (**tPA**). These results point to differences in the substrate specificity of various PDI enzymes, at least in the context of the E. coli periplasm.

L8 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:69055 CAPLUS
DOCUMENT NUMBER: 114:69055
TITLE: Pharmaceutical composition comprising a plasminogen activator and hirudin
INVENTOR(S): Heim, Jutta; Agnelli, Giancarlo; Czendlik, Czeslaw
PATENT ASSIGNEE(S): Ciba-Geigy A.-G., Switz.; UCP Gen-Pharma A.-G.
SOURCE: Eur. Pat. Appl., 33 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 365468 | A1 | 19900425 | EP 1989-810676 | 19890912 |
| EP 365468 | B1 | 19940216 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| AT 101524 | E | 19940315 | AT 1989-810676 | 19890912 |
| AU 8941352 | A1 | 19900329 | AU 1989-41352 | 19890913 |
| AU 628854 | B2 | 19920924 | | |
| US 5126134 | A | 19920630 | US 1989-408836 | 19890918 |
| CA 1336493 | A1 | 19950801 | CA 1989-611903 | 19890919 |
| DK 8904639 | A | 19900322 | DK 1989-4639 | 19890920 |
| JP 02121934 | A2 | 19900509 | JP 1989-242356 | 19890920 |
| ZA 8907173 | A | 19900926 | ZA 1989-7173 | 19890920 |
| IL 91706 | A1 | 19970713 | IL 1989-91706 | 19890920 |
| PRIORITY APPLN. INFO.: | | | GB 1988-22147 | 19880921 |
| | | | EP 1989-810676 | 19890912 |

AB Pharmaceutical compns. contg. a plasminogen activator and a hirudin can be used for prophylaxis and therapy of thrombosis or diseases caused by thrombosis. The dissoln. of thrombi is accelerated significantly and the risk of reocclusion is considerably reduced when using this combination rather than a plasminogen activator alone. Lysis of thrombi were obsd. with a rabbit jugular vein thrombosis model; i.v. administration of **tissue plasminogen activator** (**tPA**) alone, of **tPA** and heparin, and of **tPA** and hirudin produced 37-44, 34, and 52% clot lysis, resp. Addnl., the presence of hirudin decreased thrombin accretion by approx. 50% relative to **tPA** alone or to **tPA** and heparin. Expression vectors for manuf. of hirudin mutants in Escherichia coli and yeast were prepd.

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# WEST Search History

DATE: Thursday, August 28, 2003

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| *DB=USPT,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | | |
| L7 | L4 and pComb3Hss | 0 | L7 |
| L6 | L4 and pComb3? | 8 | L6 |
| L5 | L4 and pComb? | 33 | L5 |
| L4 | phagemid | 4338 | L4 |
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| L3 | pcomb3hss | 1 | L3 |
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| L1 | ('5840533')[PN] | 1 | L1 |

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☐ 1. Document ID: US 6610512 B1

L6: Entry 1 of 8 File: USPT Aug 26, 2003

US-PAT-NO: 6610512
DOCUMENT-IDENTIFIER: US 6610512 B1

TITLE: Zinc finger binding domains for GNN

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Barbas; Carlos F. | Solana Beach | CA | | |

US-CL-CURRENT: 435/69.1; 530/350, 536/23.1

ABSTRACT:

Zinc finger-nucleotide binding polypeptides having binding specificity for target nucleotides containing one or GNN triplets are provided. Compositions containing such polypeptides and the use of such polypeptides and compositions for regulating gene expression are also provided.

7 Claims, 8 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6538114 B1

L6: Entry 2 of 8 File: USPT Mar 25, 2003

US-PAT-NO: 6538114
DOCUMENT-IDENTIFIER: US 6538114 B1

TITLE: Human monoclonal antibodies specific for hepatitis C virus (HCV) E2 antigen

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Persson; Mats Axel Atterdag | Stockholm | | | SE |
| Allander; Tobias Erik | Stockholm | | | SE |

US-CL-CURRENT: 530/388.3; 424/130.1, 424/133.1, 424/139.1, 424/141.1, 424/147.1, 424/149.1, 435/455, 435/5, 435/69.1, 435/7.1, 530/388.1

ABSTRACT:

The present invention relates to compositions derived from immunoglobulin molecules specific for the hepatitis C virus (HCV). More particularly, the invention is related to molecules which are capable of specifically binding with HCV E2 antigen. The molecules are useful in specific binding assays, affinity purification schemes and pharmaceutical compositions for the prevention and treatment of HCV infection in mammalian subjects. The invention thus relates to novel human monoclonal antibodies specific for HCV E2 antigen, fragments of such monoclonal antibodies, polypeptides having structure and function substantially homologous to antigen-binding sites obtained from such monoclonal antibodies, nucleic acid molecules encoding those polypeptides, and expression vectors comprising the nucleic acid molecules.

46 Claims, 17 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 9

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

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☐ 3. Document ID: US 6491894 B1

L6: Entry 3 of 8 File: USPT Dec 10, 2002

US-PAT-NO: 6491894
DOCUMENT-IDENTIFIER: US 6491894 B1

TITLE: NGR receptor and methods of identifying tumor homing molecules that home to angiogenic vasculature using same

DATE-ISSUED: December 10, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Ruoslahti; Erkki | Rancho Santa Fe | CA | | |
| Pasqualini; Renata | Solana Beach | CA | | |

US-CL-CURRENT: 424/9.1; 424/9.2, 424/93.2, 435/7.23, 435/7.8, 436/501, 514/2, 530/300

ABSTRACT:

The present invention provides a method of identifying a tumor homing molecule that homes to angiogenic vasculature by contacting a substantially purified NGR receptor with one or more molecules and determining specific binding of a molecule to the NGR receptor, where the presence of specific binding identifies the molecule as a tumor homing molecule that homes to angiogenic vasculature. The invention also provides a method of directing a moiety to angiogenic vasculature in a subject by administering to the subject a conjugate including a moiety linked to a tumor homing molecule that exhibits specific binding to an NGR receptor, whereby the moiety is directed to angiogenic vasculature. In addition, the invention provides a method of imaging the angiogenic vasculature of a tumor in a subject by administering to the subject a conjugate having a detectable moiety linked to a tumor homing molecule that exhibits specific binding to an NGR receptor and detecting the conjugate.

3 Claims, 49 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 16

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 4. Document ID: US 6395275 B1

L6: Entry 4 of 8 File: USPT May 28, 2002

US-PAT-NO: 6395275
DOCUMENT-IDENTIFIER: US 6395275 B1

TITLE: Synthetic human neutralizing monoclonal antibodies to human immunodeficiency virus

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Barbas; Carlos F. | San Diego | CA | | |
| Burton; Dennis R. | La Jolla | CA | | |
| Lerner; Richard A. | La Jolla | CA | | |

US-CL-CURRENT: 424/148.1; 424/160.1, 435/975

ABSTRACT:

The present invention describes synthetic human monoclonal antibodies that immunoreact with and neutralize human immunodeficiency virus (HIV). The synthetic monoclonal antibodies of this invention exhibit enhanced binding affinity and neutralization ability to gp120. Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell lines for producing the monoclonal antibodies.

3 Claims, 22 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 18

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 6261558 B1

L6: Entry 5 of 8 File: USPT Jul 17, 2001

US-PAT-NO: 6261558
DOCUMENT-IDENTIFIER: US 6261558 B1

TITLE: Synthetic human neutralizing monoclonal antibodies to human immunodeficiency virus

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Barbas; Carlos F. | San Diego | CA | | |
| Burton; Dennis R. | La Jolla | CA | | |
| Lerner; Richard A. | La Jolla | CA | | |

US-CL-CURRENT: 424/133.1; 424/134.1, 424/135.1, 424/142.1, 424/148.1, 424/160.1, 424/188.1, 424/208.1, 435/252.3, 435/252.33, 435/320.1, 435/328, 435/339.1, 435/402, 435/403, 435/440, 435/5, 435/69.6, 435/69.7, 435/70.21, 435/91.1, 530/387.3, 530/388.15, 530/388.35, 536/23.53

ABSTRACT:

The present invention describes synthetic human monoclonal antibodies that immunoreact with and neutralize human immunodeficiency virus (HIV). The synthetic monoclonal antibodies of this invention exhibit enhanced binding affinity and neutralization ability to gp120. Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell lines for producing the monoclonal antibodies.

40 Claims, 22 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 18

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 6. Document ID: US 6255455 B1

L6: Entry 6 of 8 File: USPT Jul 3, 2001

US-PAT-NO: 6255455
DOCUMENT-IDENTIFIER: US 6255455 B1

TITLE: Rh(D)-binding proteins and magnetically activated cell sorting method for production thereof

DATE-ISSUED: July 3, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Siegel; Donald L. | Hatboro | PA | | |

US-CL-CURRENT: 530/350; 435/5, 435/6, 435/7.1, 435/7.21, 435/7.25, 530/380, 530/386, 530/387.1

ABSTRACT:

The invention includes Rh(D) binding proteins, including antibodies, and DNA encoding such proteins. Methods of generating such proteins and DNAs are also included.

21 Claims, 47 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 7. Document ID: US 6180084 B1

L6: Entry 7 of 8 File: USPT Jan 30, 2001

US-PAT-NO: 6180084
DOCUMENT-IDENTIFIER: US 6180084 B1

TITLE: NGR receptor and methods of identifying tumor homing molecules that home to angiogenic vasculature using same

DATE-ISSUED: January 30, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Ruoslahti; Erkki | Rancho Santa Fe | CA | | |
| Pasqualini; Renata | Solana Beach | CA | | |

US-CL-CURRENT: 424/9.1; 424/9.2, 435/7.8, 436/501

ABSTRACT:

The present invention provides a method of identifying a tumor homing molecule that homes to angiogenic vasculature by contacting a substantially purified NGR receptor with one or more molecules and determining specific binding of a molecule to the NGR receptor, where the presence of specific binding identifies the molecule as a tumor homing molecule that homes to angiogenic vasculature. The invention also provides a method of directing a moiety to angiogenic vasculature in a subject by administering to the subject a conjugate including a moiety linked to a tumor homing molecule that exhibits specific binding to an NGR receptor, whereby the moiety is directed to angiogenic vasculature. In addition, the invention provides a method of imaging the angiogenic vasculature of a tumor in a subject by administering to the subject a conjugate having a detectable moiety linked to a tumor homing molecule that exhibits specific binding to an NGR receptor and detecting the conjugate.

3 Claims, 12 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 16



☐ 8. Document ID: US 6140081 A

L6: Entry 8 of 8 File: USPT Oct 31, 2000

US-PAT-NO: 6140081
DOCUMENT-IDENTIFIER: US 6140081 A
**** See image for Certificate of Correction ****

TITLE: Zinc finger binding domains for GNN

DATE-ISSUED: October 31, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Barbas; Carlos F. | Del Mar | CA | | |

US-CL-CURRENT: 435/69.1; 435/252.2, 435/320.1, 435/325, 514/2, 514/44, 530/350, 536/23.1

ABSTRACT:

Zinc finger-nucleotide binding polypeptides having binding specificity for target nucleotides containing one or GNN triplets are provided. Compositions containing such polypeptides and the use of such polypeptides and compositions for regulating

nucleotide function are also provided.

45 Claims, 6 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6



| Terms | Documents |
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